

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Docket No: Q87778

Jaume Ribas PINOL, et al.

Appln. No.: 101535,416

Group Art Unit: 1645

Confirmation No.: 7473

Examines: Khatol S SHAHNAN SHAH

Filed: May 19,2005

For: LIVE ATTENUATED VACCINE AGAINST PORCINE PLEUROPNEUMONIA"

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Laura Ferrer Soler, hereby declare and state:
2. THAT I am a citizen of Spain;
3. THAT I have received the degree of Ph. D. in Biology in 2007 from Faculty of Health Sciences, University of Girona;
4. THAT I have been employed by Laboratorios HIPRA, S.A. since 2006, where I hold a position as R+D researcher, with responsibility for coordinating new biological products projects;
5. THAT I am familiar with the disclosure and claims of the above-identified patent application.
6. I am familiar with the prosecution of this application. I have reviewed the Office Action dated February 13,2008, in the above-identified application wherein, infer

alia, the Examiner rejects claims 19 under 35 U.S.C. § 112, first paragraph, for lack of enablement.

7. THAT the following Declaration is provided for the purpose of providing data to show the efficacy and usefulness of the claimed vaccine against *Actinobacillus pleuropneumoniae* caused by porcine pleuropneumonia.

A live vaccine against porcine pleuropneumonia comprising an immunogenic and non-haemolytic *Actinobacillus pleuropneumoniae* (App) strain (CECT 5994)

I present a live, attenuated vaccine composition comprising an immunogenically effective amount of an *Actinobacillus pleuropneumoniae* (App) serotype 1 strain formulated in a pharmaceutically acceptable carrier. The strain used in the vaccine composition comprises a mutation – a deletion – in the segment of gene *apxIA* which codes the second transmembrane domain of the exotoxin ApxI of App. Additionally, a deletion in the segment of *apxIIA* gene which codes the second transmembrane domain of the ApxII exotoxin of App is also introduced.

As a result, the live attenuated vaccine prepared with this App modified strain, contains ApxI and ApxII toxins without haemolytic activity but maintains unaltered its immunoprotective ability because it contains all the antigens immunologically necessary to obtain a high immunogenic response in the host. Therefore, the App strain obtained is an immunogenic strain without haemolytic activity.

The immunogenic and non-haemolytic strain of *Actinobacillus pleuropneumoniae* used for formulating this live attenuated vaccine corresponds to the App strain deposited in the

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Colección Española de Cultivos Tipo (Spanish Collection of Type Cultures) with the registration number CECT 5994, according to the Budapest Treaty (as disclosed at page 4 lines 15-18 and page 13, lines 16-28 of the specification).

This strain has shown to be suitable to prepare a live attenuated vaccine against porcine pleuropneumonia. The results show that the method object of the present invention gives attenuated App strains able to generate a potent immune response and also to produce non-haemolytic ApxI and ApxII toxins. Therefore, the attenuated App strain induces a potent immune response without but bereft of the lesions and the signs caused by an infection with a pathogenic App. To date, this represents a significant improvement over the App attenuated strains used as vaccine candidate.

The immune response induced by live vaccines is different than that induced by inactivated vaccines and lasts longer. The live attenuated vaccines described in the state of the art, based on App strains without haemolytic capability, are less immunoprotective because they have suffered modifications in their structure that do not allow them to attach to the membrane receptor of the target cells. Furthermore, these can not induce antibodies against ApxI and/or ApxII toxins, since these are not secreted by the cell. Additionally, the live attenuated antigen strain CECT 5994 is capable of expressing its own exotoxins but without haemolytic activity. These exotoxins constitute the most important virulence factors against which the host animal addresses the immune response. In that way the immune response against these exotoxins would not be limited to that induced by antigen strains belonging to the same serotype, but to the wide range of serotypes classified within the same toxigenic group (group of strains that produce the

same exotoxins). Therefore the protection induced by one live vaccine would not be serotype but toxigenic group specific.

I have conducted different studies to assess the efficacy of the vaccines of the present invention.

The following table shows the scheme used to demonstrate the efficacy of a live vaccine against porcine pleuropneumonia comprising an immunogenic and non-haemolytic App strain (CECT 5994). The strain was obtained by the method described in claims 21-29 of the present application. The live attenuated strain was formulated in a pharmaceutically aqueous vehicle and directly inoculated to different groups of animals.

In the efficacy study, the lung lesion reduction was compared between groups vaccinated or not vaccinated with a serotype 1 vaccine (CECT 5994 strain) and challenged with the homologous App serotype 1 strain. Moreover, a vaccinated and non infected group was also included in order to test vaccine safety. 3 groups of 10 animals were vaccinated at 8 and 11 weeks of age, and challenged 3 weeks after second vaccination following this scheme:

GROUP	VACCINE	CHALLENGE
1	non vaccinated	homologous serotype 1
2	vaccinated	homologous seiotype 1
3	vaccinated	non infected

Necropsy and lung lesion assessment were performed on day 7 post-challenge according to the Pharm. Eur. monograph for *Actinobacillus pleuropneumoniae* vaccines. For lung lesion evaluation the following criteria was applied: lung was divided in 7 parts (apical right/left,

intermediate right/left, diaphragmatic right/left, cardiac) and a score from 0 to 5 was assigned according to the affected area (0: no affectation, 5: maximum affectation). Results are shown in Figure 1.

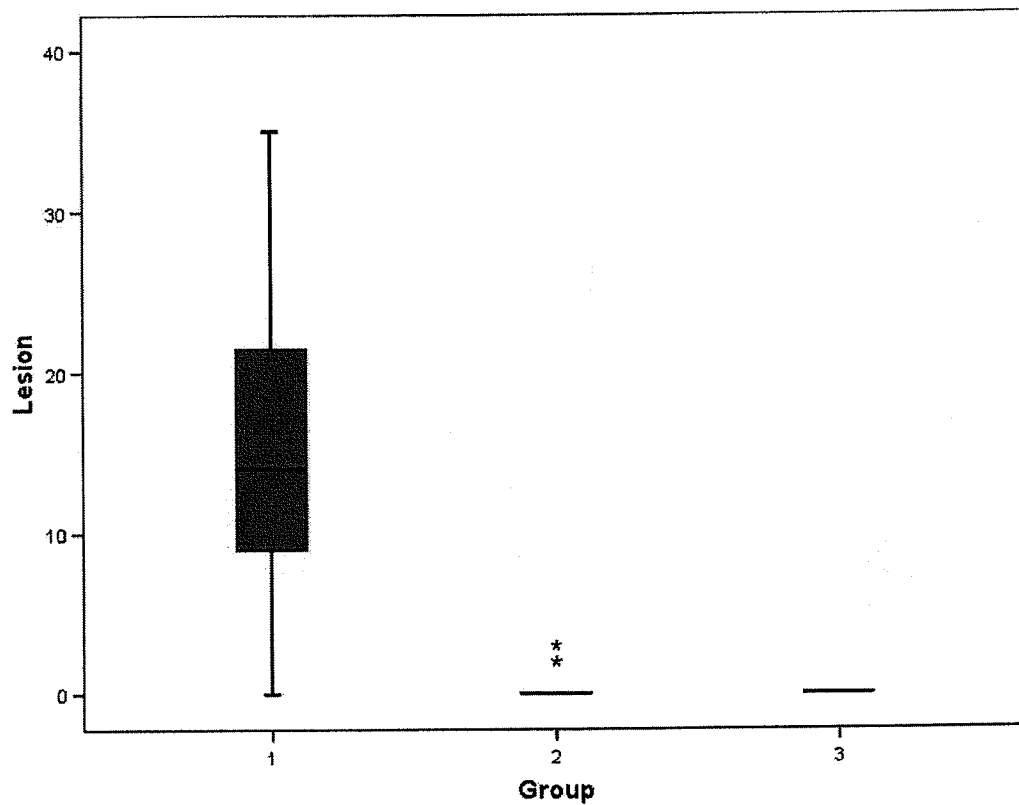


Figure 1. Lung lesion score of animals from different groups (non-vaccinated and infected with homologous App serotype 1 (group 1), vaccinated and infected with homologous App serotype 1 (group 2), and vaccinated and non infected (group 3).

Lung lesion score was compared using non parametric Mann-Whitney test. Results are shown in Table 1.

Group	Percentage of animals with low lung lesion level (from 0 to 7)	Percentage of lung lesion score reduction compar non vaccinated group	significance
1	18		
2	100	82	significant
3	100		

0.000
(p<0.05)

Mann-Whitney (non parametric)				
Comparative (groups)	U Mann-Whitney	W Wilcoxon	Z	Sign. asint. bil.
1 and 2	7	85	-3,846	0,000

significant

Table 1. Statistical **analysis** of the comparative between groups (non vaccinated and vaccinated) and **infected**. Statistical test applied was Mann-Whitney for non **parametric samples**. **Percentage of lung lesion** score reduction was calculated **subtracting** non vaccinated percentage of **animals with low lung lesion level** to vaccinated **percentage of animals with low lung lesion level**.

As can be observed in Figure 1 and Table 1, the vaccinated and infected group (group 2) had a significant reduction in lung lesion injuries when compared to the non-vaccinated and

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infected group (group 1). This reduction was of 82%. Group 3, the vaccine safety group, showed no lesions in any of the lungs analysed. This confirms the safety of the attenuated immunogenic and non-haemolytic strain.

The vaccinal strain here presented is demonstrated to be able to generate an immune response due to the fact it is a live, attenuated vaccine and also to produce non-haemolytic ApxI and ApxII toxins. The key factor is that the exotoxins that this strain expresses are attenuated and so they are not able to cause hemorrhagic lesions. On the other hand, the toxins are able to stimulate an immune response which is needed to battle the infection. Figure 2 shows representative lungs from each group. Typical hemorrhagic pleuropneumonia lesions caused by App are marked with a circle.

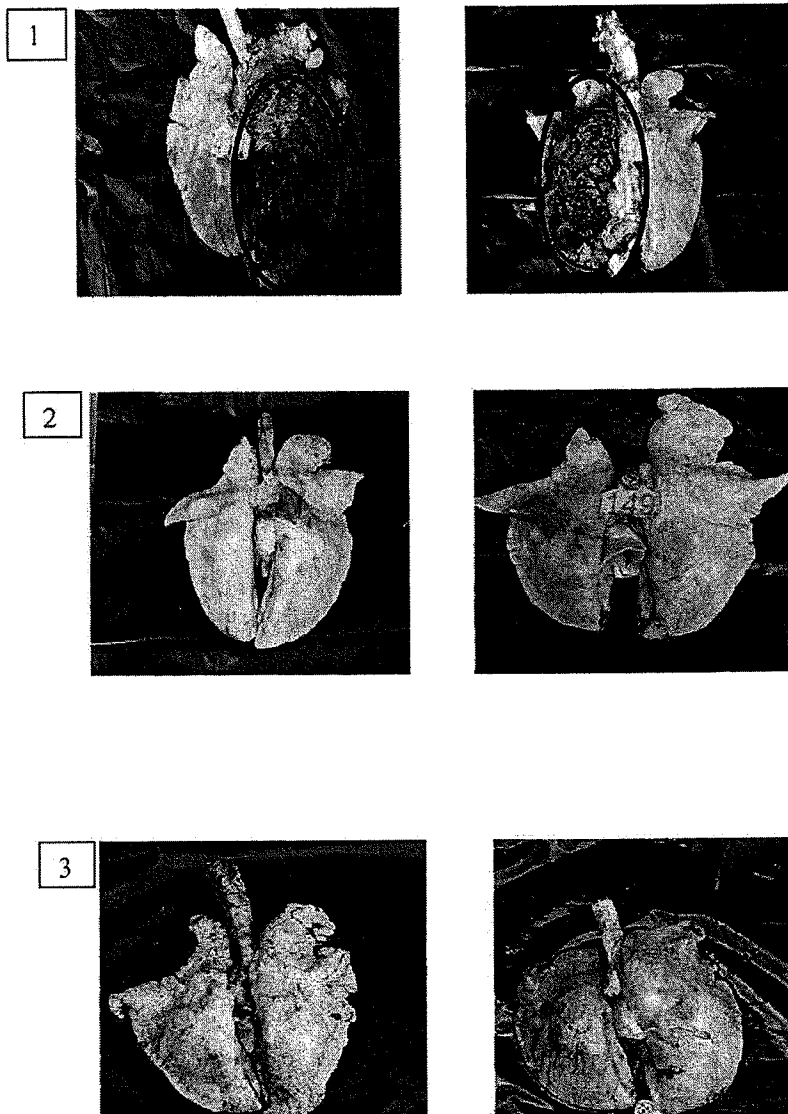


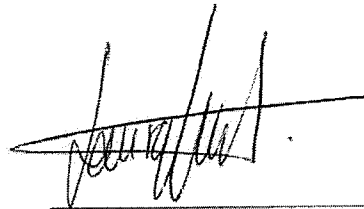
Figure 2. Lungs of animals from different groups. (1) non vaccinated and infected with homologous App serotype 1, (2) vaccinated and infected with homologous App serotype 1, and (3) vaccinated and non infected. Typical hemorrhagic lesions caused by App are marked with a black circle.

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As shown in the experimental results, the strains of the present invention are immunogenic and attenuated and therefore can be used as vaccine strains to prevent App induced lesions and clinical signs. This invention represents a significant step forward in vaccine development for the protection of swine against porcine pleuropneumonia infections.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 04/06/08



Dr. Laura Ferrer Soler